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Leptin and inflammation-associated cachexia in chronic kidney disease

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Leptin is an adipocyte-derived hormone that acts as a major regulator of food intake and energy homeostasis. It circulates both as a free and as a protein-bound entity. Leptin is released into the blood in proportion to the amount of body fat and exerts sustained inhibitory effects on food intake while increasing energy expenditure. The leptin receptor belongs to the class I cytokine receptor superfamily and possesses strong homology to the signal-transducing subunits of the IL-6 receptor. The hypothalamic melanocortin system, and specifically the melanocortin-4 receptor (MC-4R), is critical in mediating leptin's effect on appetite and metabolism. Serum leptin concentrations are elevated in patients with chronic kidney disease (CKD) and correlate with C-reactive protein levels suggesting that inflammation is an important factor that contributes to hyperleptinemia in CKD. Hyperleptinemia may be important in the pathogenesis of inflammation-associated cachexia in CKD. We showed that experimental uremic cachexia was attenuated in *db/db* mice, a model of leptin receptor deficiency. Nephrectomy in these animals did not result in any change in weight gain, body composition, resting metabolic rate, and efficiency of food consumption. Furthermore, experimental uremic cachexia could be ameliorated by blocking leptin signaling through the hypothalamic MC-4R. MC-4R knockout mice or mice administered the MC-4R and MC-3R antagonist, agouti-related peptide, resisted uremia-induced loss of lean body mass and maintained normal basal metabolic rates. Thus, melanocortin receptor antagonism may provide a novel therapeutic strategy for inflammation-associated cachexia in CKD.

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A decade ago, Friedman and colleagues identified by positional cloning an obese (OB) gene that is responsible for obesity in the *ob/ob* mouse.[1] This discovery has initiated a new era of research on molecular mechanisms of energy homeostasis. The *OB* gene encodes a 16-kDa circulating hormone, named leptin after the Greek word '*leptos*', meaning lean. Leptin is an adipocyte-derived hormone that acts as a major regulator of food intake and energy homeostasis. It circulates both as a free and as a protein-bound entity. Structural studies demonstrate leptin as a member of the growth hormone four-helical cytokine subfamily. Comparative structural analysis results in a phylogeny that positions leptin in a subfamily that includes growth hormone, erythropoietin, leukemia inhibitory factor, and interleukins (IL) 2, 3, 4, 5, 6, and 10. Specifically, within this structural family, leptin resides in the middle of the long- and short-chain helical members, closest to IL-6.[2]

LEPTIN RECEPTORS

Tartaglia *et al.*[3] identified the leptin receptor gene in the db locus of the mouse chromosome 4. The leptin receptor belongs to the class I cytokine receptor superfamily. The extracellular leptin-binding domain of the leptin receptor possesses strong homology to the gp130 signal-transducing subunits of receptor for IL-6 and leukemia inhibitory factor. All of these receptors, including the leptin receptor, couple to the Janus kinase/signal transducer and activator of transcription signal transduction pathway. At least five isoforms (OBRa, OBRb, OBRc, OBRd, and OBRe) are known to exist and result from alternate gene splicing.[4] All leptin receptors share an identical N-terminal ligand-binding domain but differ at the C-terminal region. The OBRa, OBRb, OBRc, and OBRd receptor isoforms contain a single transmembrane region, whereas the OBRe receptor is truncated proximal to the membrane-spanning domain. This last receptor isoform without a membrane anchor functions as a circulating soluble leptin-binding receptor (SLR) protein.[5] *OBRb* is primarily expressed in the hypothalamus, where its action is important in the regulation of energy homeostasis. *OBRb* expression is also detected in a large number of peripheral tissues including skeletal muscle, heart, adrenals, kidneys, adipocytes, immune cells, liver, and pancreatic cells. Thus, leptin may have a wide spectrum of peripheral functions as

well.[6] The functions of other isoforms of the leptin receptor with shortened cytoplasmic domains (OBRa, OBRc, OBRd) have yet to be determined. These receptors are abundantly expressed in most tissues and have been suggested to function in leptin clearance or to facilitate transport into the central compartment.[7,8]

LEPTIN TRANSPORT AND SENSITIVITY

There is evidence to suggest that central leptin sensitivity may be related to its transport across the blood–brain barrier. In obese humans, who are leptin resistant, the ratio of leptin in cerebrospinal fluid to plasma is decreased, indicating that the capacity for leptin transport into the brain is reduced. This apparent reduction in leptin transport into the central nervous system may be a significant cause of leptin resistance in obesity.[9] Rodents with obesity from overfeeding do not lose weight when leptin is administered peripherally but respond robustly to leptin when it is given intracerbroventrically.[10] Leptin is transported across the blood–brain barrier in mice in which a nonsense mutation in the extracellular domain of the leptin receptor deletes all functional receptor isoforms.[11] However, the exact nature of the putative leptin transporter and its physiological regulation remain to be determined.

LEPTIN ACTION ON APPETITE AND ENERGY HOMEOSTASIS

Leptin is released into the blood in proportion to the amount of body fat and exerts sustained inhibitory effects on food intake while increasing energy expenditure. When body fat stores fall, declining levels of leptin are sensed by the brain and are transduced into increases in appetite and metabolic efficiency that persist until the lost weight is recovered. In addition, short-duration, meal-related 'satiety' signals, such as cholecystokinin and peptide YY, are released from the gastrointestinal tract during eating. These peptides promote a sense of 'fullness' that encourages an end to the meal. Ghrelin is another gut peptide that increases with fasting and is thought to trigger the onset of eating by stimulating appetite. Collectively, these hormones participate in a meal-to-meal control system that is itself sensitive to changes in insulin and leptin levels. In this way, the size and frequency of individual meals can be adjusted so as to minimize changes in body fat content.[12]

The melanocortin system is critical in mediating leptin's effect on metabolism and that there are distinct local counterparts of the pro-opiomelanocortin (POMC) cells: agouti-related protein (AgRP) and neuropeptide Y (NPY) producing cells in the arcuate nucleus. Activation of POMC neurons by leptin triggers the release of α-melanocyte-stimulating hormone from POMC axon terminals, which in turn activates the type 4 melanocortin receptor (MC-4R), leading to suppressed food intake and increased energy expenditure. Simultaneously, leptin suppresses the activity of arcuate nucleus NPY/AgRP neurons, which otherwise would antagonize the effect of α-melanocyte-stimulating hormone (α-MSH) on MC-4Rs through the release of AgRP. Not only does the NPY/AgRP system antagonize anorexigenic melanocortin cells at their target sites, where MC-4Rs are located, but it also very robustly and directly inhibits POMC perikarya through both NPY and the inhibitory neurotransmitter GABA, which acts through basket-like synaptic innervation of POMC cells by NPY/AgRP cell terminals. This apparent unidirectional anatomical interaction between the NPY/AgRP and POMC perikarya is of potential significance, as it provides a tonic inhibition of the melanocortin cells whenever the NPY/AgRP neurons are active.[13,14]

Both NPY/AgRP and POMC neuronal systems are directly targeted by leptin and can also be affected by other peripheral metabolic signals, such as ghrelin, glucose, insulin and peptide YY, a putative satiety signal released from the gastrointestinal tract postprandially in proportion to the calorie content of a meal.[15] Their localization in ventromedial aspects of the arcuate nucleus close to the median eminence, a region with an attenuated blood–brain barrier between vessels and the parenchyma, means that these neurons might be reached by circulating metabolic signals in the most effective manner. Indeed, NPY/AgRP or POMC perikarya or dendrites are often seen in direct contact with capillaries, thus increasing the likelihood that these neurons are direct targets of peripheral signals.[13,14]

LEPTIN ACTION AS AN INFLAMMATORY CYTOKINE

Shamsuzzaman *et al.*[16] demonstrated an association between serum levels of leptin and C-reactive protein (CRP) in healthy young adults. The investigators postulate that leptin correlates with CRP either because leptin directly stimulates the production of IL-6 or because obese patients tend to produce large quantities of leptin and IL-6. Ble *et al.*[17] used data from a large population-based epidemiology study of older subjects to show that serum levels are directly associated with CRP and that this association is independent of IL-6, other cytokines, and a number of potentially confounders such as adiposity, lifestyle, drugs, and diseases. Treatment of overweight men with a leptin analogue during a low-calorie diet prevented the CRP decrease usually observed during weight loss, without affecting IL-6 levels.[18] Leptin directly stimulates the production of acute-phase proteins such as CRP in liver cells.[19] Both leptin and IL-6 receptors are expressed on hepatocyte cell surface.[3,20] Leptin receptors can activate intracellular Janus kinase/signal transducer and activator of transcription signaling pathways normally activated by IL-6.[20] It is possible that these shared pathways explain the similarity of effects between leptin and IL-6 on CRP production. As a cytokine, leptin appears to serve in thymic homeostasis and to contribute to the regulation of immune function.[6]

LEPTIN IN CHRONIC KIDNEY DISEASE

Serum leptin concentrations are elevated in chronic kidney disease (CKD). The mechanism underlying elevated serum leptin levels in patients with CKD differs from that in obese

patients. Leptin is cleared from the circulation by the kidney by glomerular filtration followed by metabolic degradation in the renal tubules.[21] In patients with normal renal function, there is a net renal intake of 12% of circulating leptin, whereas in patients with CKD, there is no renal update of leptin.[22] In hemodialysis patients, serum leptin levels factored for body mass index was increased by fourfold compared with healthy controls. Serum leptin is not effectively cleared by hemodialysis with cellulose membranes.[22] Furthermore, leptin release from adipose tissue is decreased by metabolic acidosis.[23] Thus, the presence of metabolic acidosis in end-stage renal disease patients may partially mask their hyperleptinemia. In a cross-sectional study of 219 patients with various degrees of CKDs, significant correlations were found between leptin levels and glomerular filtration rate and body mass index. Leptin gene expression in adipose tissue from CKD patients was decreased. Thus, decreased plasma leptin clearance in CKD may be part of an efferent feedback loop that downregulates the expression of the *OB* gene in hyperleptinemic patients with advanced CKD. Furthermore, a significant correlation between leptin and CRP concentrations were also demonstrated in CKD patients, suggesting that inflammation is an important factor that contributes to hyperleptinemia in CKD.[24] Longitudinal studies showed that peritoneal dialysis (PD) patients who were cachexia, with loss of lean body mass during the observation period, had higher initial CRP levels. A significant increase in serum leptin concentrations was observed in PD patients who lost lean body mass, whereas no such change was observed in PD patients who gained lean body mass. This suggests that hyperleptinemia may be an important cause of uremic cachexia.[25]

Pecoits-Filho *et al.*[26] studied 149 non-obese end-stage renal disease patients in order to analyze the association between serum leptin, SLR, inflammation, and body composition. These investigators found significantly elevated serum leptin concentrations but normal SLR concentrations in the end-stage renal disease patients compared with healthy controls with normal renal function. A negative correlation was observed between serum leptin and SLR in the end-stage renal disease patients. A positive correlation was observed between lean body mass and the SLR/serum leptin ratio. Fat mass was negatively correlated with both SLR and SLR/serum leptin ratio. Furthermore, a positive correlation was found between IL-6 and serum leptin concentrations. These results suggest that free circulating bioactive leptin concentrations are elevated in patients with end-stage renal disease and may be associated with inflammatory-associated cachexia.[26] Voegeling and Fantuzzi[27] showed that inflammation induced by lipopolysaccharide injections is associated with a very significant increase in serum leptin and only a marginal increase in SLR concentrations, again suggesting that free circulating bioactive leptin concentrations are present in this model of infection-associated cachexia. Furthermore, Huang *et al.*[28] demonstrated that lipopolysaccharide-induced anorexia in rats can be reversed by a central melanorcortin antagonist. Indeed, Marks *et al.*[29] have shown more recently that cachexia induced by lipopolysaccharide and tumor growth can be ameliorated by blocking leptin signaling through the hypothalamic MC-4R. MC-4R knockout (KO) mice or mice administered the MC-4R and MC-3R antagonist, AgRP, resist tumor-induced loss of lean body mass and maintain normal basal metabolic rates during tumor growth[29] (Figure 1).

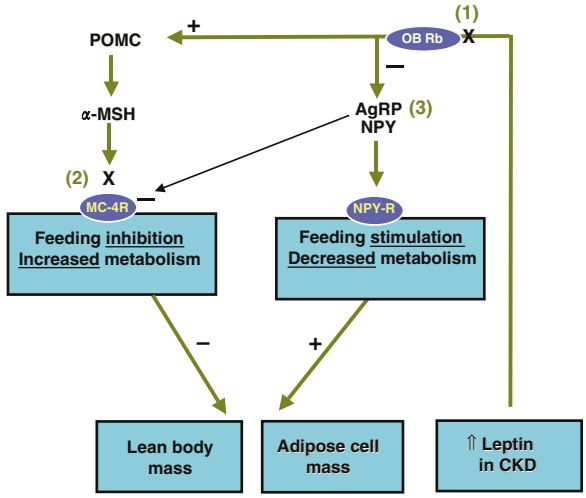

Figure 1 | Experimental strategies for treatment of uremic cachexia (Cheung *et al.*[30]). (1) Leptin receptor (OBRb) knockout. (2) MC-4R knockout. (3) AgRP, antagonist for MC-4R and MC-3R.

LEPTIN AND MELANOCORTIN SIGNALING IN UREMIC CACHEXIA

We tested the hypothesis that leptin was an important cause of uremic cachexia via signaling through its receptor.[30] Our results showed that uremic cachexia was attenuated in *db/db* mice, a model of leptin receptor deficiency. Nephrectomy in these animals did not result in any change in weight gain, body composition, resting metabolic rate, and efficiency of food consumption. Recent studies suggested that *db/db* mice resisted lipopolysaccharide-induced anorexia by reducing tumor necrosis factor-α secretion.[31] Thus, leptin may have an important role in the regulation of appetite, body composition, and metabolic rate in uremia. Indeed, elevated serum leptin was associated with lower dietary intake and higher catabolic rate in uremic children.[32]

We demonstrated that uremic cachexia in experimental animals can be attenuated by blocking leptin signaling through the central MC-4R. Both homozygous and heterozygous MC-4RKO mice had no decrease in appetite after nephrectomy compared with wild-type sham animals. The most striking difference was that both the homozygous and heterozygous MC-4RKO animals continued to gain lean body mass and fat mass with no change in the food consumption efficiency despite the cachexic effects of uremia as demonstrated in the wide-type nephrectomized controls. The effects of nephrectomy on increasing resting metabolic rate as demonstrated in the wild-type animals were also much

attenuated in both the homozygous group and the heterozygous group. These results are consistent with previous observations that MC-4RKO animals maintained normal metabolic rate and body composition, even when bearing a carcinoma that produced classic cachexia in wild-type control animals.[29] These data strongly suggest that the hypothalamic MC-4R plays a significant role in transducing cachexigenic signals in uremia.

We further tested the effect of central melanocortin receptor antagonism in the experimental uremic cachexia models using a pharmacological approach. These data clearly demonstrated that uremic cachexia is ameliorated by central administration of AgRP in wild-type nephrectomized mice. Repeated intracranial infusion of AgRP significantly increased food intake, weight gain including both lean and fat mass, as well as efficiency of food consumption. At the same time, resting metabolic rate was decreased by AgRP. Circulating leptin concentrations were elevated in wild-type nephrectomized mice but normalized in AgRP-treated wild-type nephrectomized mice. These data are consistent with the role of AgRP as an antagonist for MC-4R and with previous studies demonstrating that tumor-induced and sepsis-induced cachexia is attenuated by MC-4R blockade with AgRP.[30]

CONCLUSION

Leptin signaling, through the hypothalamic melanocrotin receptors, may play an important role in the pathogenesis of inflammation-associated cachexia in CKD. Elucidation of the mechanisms regulating appetite and metabolic rate will permit new insights into the pathophysiology of uremic cachexia. We recently showed that experimental uremic cachexia was attenuated in *db/db* mice, a model of leptin receptor deficiency. Nephrectomy in these animals did not result in any change in weight gain, body composition, resting metabolic rate, and efficiency of food consumption. Furthermore, MC-4RKO mice or mice administered the MC-4R and MC-3R antagonist, AgRP, resisted uremia-induced loss of lean body mass and maintained normal basal metabolic rates. These recent findings increase our understanding of the pathogenesis and may provide the basis of a novel therapeutic strategy for inflammation-associated cachexia in CKD.

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